FINAL TECHNICAL REPORT

NASA Grant NAGW - 349

Changes in Bone Structure and Metabolism during Simulated Weightlessness: Endocrine and Dietary Factors

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I. Technica' Report

The overall objective of this grant was to examine the roles of vitamin D, PTH and corticosterone in the skeletal alterations induced by simulated weightlessness. In light of the studies by Globus et al. and others suggesting that bone formation is inhibited following acute skeletal unloading (Endo. 114:2264, 1984, Metab. Bone Dis. Rel. Res. 4169, 1982), our first objective was to determine if changes in the serum concentrations of Ca, P_i, osteocalcin, 25-OH-D, 24,25$(OH)_2$D or 1,25$(OH)_2$D a so occurred following acute skeletal unloading. To accomplish this, animals were either suspended (using the tail suspension model of Globus et al., Endo. 114:2264, 1984) or pair fed for 2, 5, 7, 10, 12 and 15 days and the serum concentrations of Ca, P_i, osteocalcin and the vitamin D metabolites measured. In addition, bone histology was examined at day 5 after suspension. Acute skeletal unloading produced a transient hypercalcemia, a significant fall in serum osteocalcin and serum 1,25$(OH)_2$D, a slight rise in serum 24,25$(OH)_2$D, but did not affect the serum concentrations of P_i or 25-OH-D (Fig. 1). At the nadir in serum 1,25$(OH)_2$D serum osteocalcin was reduced by 22%, osteoblast surface by 32% ($P < 0.05$) and longitudinal bone growth by 21% ($P < 0.01$)(Table I).

TABLE I. Effect of skeletal unloading on bone histology in the proximal tibial metaphysis.

	Control (n = 5)	5 day suspended (n = 5)
Trebecular bone volume (per cent)	14.3 ± 1.2	13.2 ± 1.5
Osteoblast surface (per cent)	21.5 ± 0.8	14.5 ± 2.5[a]
Osteoblasts/mm	14.2 ± 0.7	8.7 ± 1.7[b]
Osteoclast surface (per cent)	36.1 ± 2.1	37.7 ± 1.6
Osteoclasts/mm	5.7 ± 0.4	5.0 ± 0.6
Longitudinal bone growth (m/day)	191.5 ± 5.8	152.0 ± 7.5[c]

Data are given as mean ± SE

[a] $p < .05$, [b] $p < .025$, [c] $p < .01$, Students t-test, 2 tailed

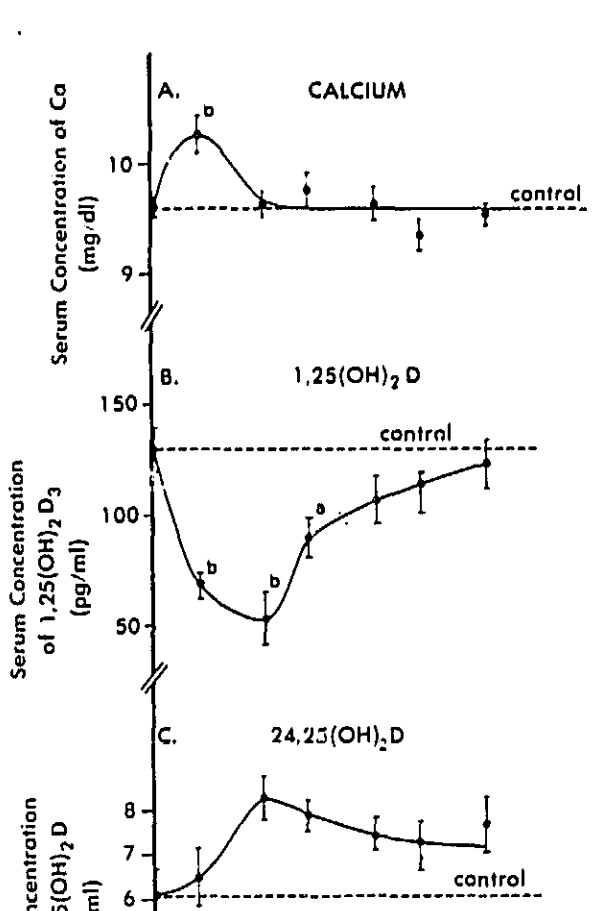

Figure 1. Effect of skeletal unloading on the serum concentrations of Ca(A), $1,25(OH)_2D$ (B) and $24,25(OH)_2D$ (C). $^aP < .05$, $^bP < .01$, Students t-test

To determine if prevention of the fall in serum $1,25(OH)_2D$ observed between days 0 and 7 of suspension would prevent the decrease in bone mineral reported by Globus et al. (Endo. 114:2264, 1984), we chronically infused rats with $1,25(OH)_2D$ so as to maintain a constant serum level of $1,25(OH)_2D$ throughout the

suspension period. Chronic $1,25(OH)_2D$ infusion did prevent the expected fall in serum $1,25(OH)_2D$ in suspended rats but did not prevent the loss of bone mineral (Fig. 2).

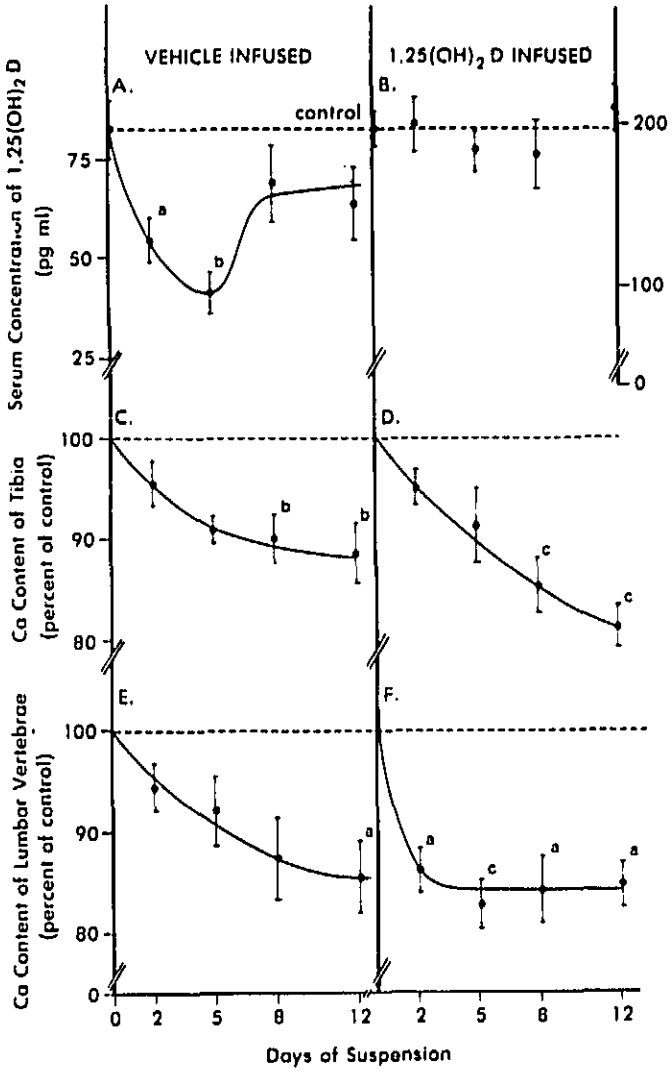

Figure 2. Effect of chronic $1,25(OH)_2D$ infusion on the serum concentration of $1,25(OH)_2D$ (A,B), and the Ca contents of the tibia (C,D) and lumbar vertebra (E,F) of suspended rats. $^{a}P < .05$, $^{b}P < .01$, $^{c}P < .005$, Students t-test

These results, taken collectively, suggest that acute skeletal unloading in the growing rat produces a transitory inhibition of bone formation which in turn produces a transitory hypercalcemia leading to a temporary decrease in serum $1,25(OH)_2D$. No evidence could be found for a direct involvement of $1,25(OH)_2D$ in the bone changes induced by skeletal unloading. On the contrary, our studies support the hypothesis that the bone loss associated with skeletal unloading is a locally mediated phenomenon and independent of vitamin D.

In a second series of studies we examined the prophylactic effect of high dietary calcium and phosphorus on the bone loss associated with acute skeletal unloading. Suspended and pair-fed rats were fed one of four diets containing the following percentages of Ca and P respectively: 0.1/0.3, 0.4/0.3, 1.2/0.8, 2.4/1.2. Animals were sacrificed after 2 weeks. Serum concentrations of Ca and PTH were unaffected by diet and skeletal unloading. Serum $1,25(OH)_2D$ increased with decreasing dietary Ca as expected, but was the same in suspended and control rats for a given diet. Total bone Ca increased in all animals as dietary Ca and P increased, and although tibial Ca in suspended rats fed the 2.4% Ca diet was equivalent to or greater than tibial Ca in control rats fed the 0.44% or 1.2% Ca diets, for each separate diet group, suspended animals had lower bone Ca values. These results suggest that high dietary Ca and P can reduce the impact of skeletal unloading on bone Ca, but can not prevent the loss of mineral.

Because of a restructuring in programs, the original studies planned to examine the role of corticosterone were not done. They are now, however, underway and under the direction of both Drs. Bernard Halloran and Daniel Bikle.

As is so often the case in basic research, two unrelated, but extremely important observations were made during the course of our $1,25(OH)_2D$ infusion studies. Animals chronically infused with $1,25(OH)_2D$ had lower serum concentrations of 25-OH-D and increased bone mass. Studies are presently underway to further examine this phenomenon.

II. Abstracts and Publications Directly Associated with this Grant

A. Abstracts

1. Halloran BP, Wronski T, Bikle DD, Holton E, Globus R, The role of PTH and $1,25(OH)_2D$ in the bone changes induced by simulated weightlessness, NASA Space Biology Symposium, p. 23, October 1983, Arlington, VA.

2. Patterson-Allen, P, Globus R, Halloran BH, Bikle D, Cann C, Morey E, Relation of serum osteocalcin and $1,25(OH)_2D$ to bone formation during skeletal unloading. Am Soc Bone Min Res, A19, June 1984, Hartford, CT.

3. Halloran BP, Bikle DD, Holton E, Levens M, Globus R, The role of vitamin D in the bone changes associated with simulated weightlessness, NASA Space Biology Symposium, p. 47, November 1984, Harpers Ferry, WV.

4. Bikle DD, Globus R, Halloran BP, Morey-Holton E, The salutary effect of dietary calcium on bone mass in a rat model of simulated weightlessness, NASA Space Biology Symposium, p. 49, November 1984, Harpers Ferry, WV.

5. Halloran BP, Bikle DD, Wronski T, Globus R, Levens MJ, Morey-Holton E, Changes in vitamin D metabolism and bone histology associated with skeletal unloading, Am Soc Bone Min Res, A105, June 1985, Washington, D.C.

B. Publications

1. Halloran BP, Bikle DD, Wronski TJ, Globus RK, Levens MJ, Morey-Holton E, The role of $1,25(OH)_2D$ in the inhibition of bone formation induced by skeletal unloading, Endocrinology (submitted).

2. Globus RK, Bikle DD, Halloran BP, Morey-Holton E, The skeletal response to dietary calcium in a rat model simulating weightlessnerss, Endocrinology (in preparation).

3. Wronski TJ, Halloran BP, Bikle DD, Globus RK, Morey E, Skeletal response to simulated weightlessness: A comparison of animal models, Av Space Envir Med (in preparation)

APPENDIX

The Role of 1,25-dihydroxyvitamin D in the Inhibition of Bone Formation Induced by Skeletal Unloading

Deleted